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THE EFFECT OF BORON DEFICIENCY ON THE GROWTH OF TOBACCO PLANTS IN AERATED AND UNAERATED SOLUTIONS[1]

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INTRODUCTION

In experiments dealing with the mineral nutrition of the tobacco plant, during the last several years, it seemed desirable to check the results in soil with those obtained in sand and water cultures. Earlier attempts to grow tobacco in water cultures, using ordinary chemically pure salts and distilled water, were unsuccessful because of a puzzling breakdown of the terminal bud.[3] This trouble developed in river-sand cultures, which were heavily leached with nutrient solutions made up with distilled water, and in unleached cultures in quartz sand (glass-blower's sand). It has also been observed in soil cultures in small containers (10-quart galvanized iron buckets) where pure chemicals were used and plants were stimulated to rapid growth. By employing tap water instead of distilled water and renewing the solutions at weekly intervals it was possible to grow tobacco plants to maturity. Further increase in growth was obtained by aerating the water cultures with compressed air bubbled through the solutions. When nitrogen gas was bubbled through the solutions of duplicate cultures no increase in growth was obtained, indicating that the beneficial action of the compressed air was not due to stirring.

The exact cause of the trouble remained unknown until the writer's attention was called to a set of tomato plants grown in boron-deficient solutions at the University of Maryland. The injuries of these tomato plants reported by Johnston and Dore (*3*)[4] were so similar to those noted on the tobacco plants that the writer felt convinced that a probable explanation of the previous failures to grow tobacco in nutrient solutions lay in a lack of boron.

The work of Warington (*6*, *7*) on the broad bean and other plants has shown that boron is essential to the normal development of certain plants. Warington (*6*) and Brenchley (*1*) gave excellent reviews of the literature bearing upon boron as an essential element for the growth of higher plants. In a recent paper these writers (*2*) suggested a relation between calcium and boron deficiency. However, as will

[1] Received for publication Dec. 6, 1928; issued April, 1929. The subject matter of this paper, with some rearrangement and additions, is essentially the same as a thesis submitted to the faculty of the graduate school of the University of Maryland in partial fulfillment of the requirements for the degree of master of science.

[2] The writer is indebted to Dr. E. S. Johnston, of the University of Maryland, for many valuable suggestions.

[3] Material from the above-mentioned cultures, which showed typical bud injury, was submitted to James Johnson, of the Office of Tobacco and Plant Nutrition, with the request that he determine whether an organism was responsible. He found no organism and considered the disease physiological.

[4] Reference is made by number (italic) to "Literature cited," p. 380.

beshown later in this paper, the symptoms of the two types of injury are not the same on the tobacco plant.

Liu (*4*) described an abnormal growth condition in tobacco in sand cultures and attributed it to excess nitrogen; but from the symptoms noted it would seem to have been caused by a lack of boron. The present writer found that the most vigorously growing plants developed the most marked boron-deficient symptoms. For this reason such injuries would apparently be most severe in the high nitrate treatments where Liu found the most vigorous growth associated with the severest injury.

Swanback (*5*) in a recent paper, which came to the writer's attention after the work reported here was practically complete, discussed the effect of boric acid on the growth of tobacco in nutrient solutions. He found that 0.4 parts per million of boron was the approximately optimum concentration and that quantities much in excess of this amount produced injury. It would seem that the concentration

FIGURE 1.—Tobacco plants placed in culture solutions November 10, 1927, series 1, (photographed December 29, 1927; scale shown in inches): A, Unaerated and no boron added; B, aerated and no boron added; C, aerated and boron added; D, unaerated and boron added

(0.5 p. p. m.) used in the work herein reported was fortunately chosen for the best results.

EXPERIMENTAL PROCEDURE

The solutions used in this work were made up on the basis of the ash analyses of the tobacco plant given by Wolff (*8*). The stock solution consisted of the following volume molecular concentrations: $Ca(NO_3)_2$, 0.1235; KNO_3, 0.0217; $Mg(NO_3)_2$, 0.0124; KH_2PO_4, 0.0422; and $MgSO_4$, 0.0125. One liter of this solution contains the approximate amounts of the corresponding elements contained in 100 gm. of dry matter of the tobacco plant. The stock solution was diluted 20 times with distilled water for growing the plants. The diluted solution contained the following chemicals, expressed as parts per million: Nitrogen (N), 205; phosphoric acid (P_2O_5), 150; potash (K_2O), 150.5; lime (CaO), 345; magnesia (MgO), 50; sulphuric acid (SO_3), 50. The distilled water used in these studies was obtained from a block-tin still and stored in 5-gallon glass bottles until used.

The cultures that received boron were supplied this element at the rate of 0.5 part per million in the form of boric acid (H_3BO_3). Manganese as manganous sulphate ($MnSO_1$, $4H_2O$) was added to all cultures at the rate of 1 part per million. Iron tartrate was added daily to all cultures at the rate of 1 c. c. of a 0.5 per cent solution per liter of culture solution.

Tobacco of the Connecticut Broadleaf variety was seeded in a flat of steam-sterilized greenhouse potting soil. At the end of one month the seedlings were transplanted into thumb pots containing the same kind of soil. After remaining in these pots for approximately one month, the roots were washed free of the soil and the plants were put into the respective nutrient solutions.

FIGURE 2.—Tobacco plants placed in culture solutions February 25, 1928, series 2, (photographed April 11, 1928; scale in inches): A, Unaerated and no boron added; B, unaerated and boron added; C, aerated and boron added; D, aerated and no boron added

Two-quart glass fruit jars were used as containers for growing the plants, which were held in position by paraffined cork supports. The jars were wrapped with two thicknesses of heavy wrapping paper to exclude most of the light from the roots. Some of the cultures were aerated by bubbling compressed air through the solutions at the rate of 1 liter an hour. A reducing valve maintained fairly constant air pressure. The nutrient solutions were never renewed because repeated renewals tend to delay the manifestation of boron-deficiency symptoms. This delay was possibly due to traces of boron in the chemicals used. Usually every other day, however, the solution in each culture jar was made up to the original volume with distilled water.

PRESENTATION AND DISCUSSION OF DATA

Plants of series 1 were put into the culture jars on November 10, 1927. On December 10 the first signs of boron deficiency appeared on the plants that had made the most vigorous growth, that is, on those in the aerated solutions. The results from this series are shown in Table 1. It is evident that boron is conducive to an increase in dry weight of plants in both the aerated and the unaerated solutions. Aeration increased growth both with and without the addition of boron. At the time of harvest, December 30, all the plants in the boron-deficient solutions of the aerated cultures exhibited recognizable symptoms of boron deficiency, whereas only four of the six plants in the unaerated cultures showed definite symptoms of injury from boron deficiency. A group of representative plants from this series is shown in Figure 1.

TABLE 1.—*Average green and air-dry weights (grams) of tobacco plants grown in aerated and unaerated nutrient solutions, with and without the addition of boron, from November 10 to December 30, 1927 (series 1)*

[Amount of boron=0.5 p. p. m.]

Treatment	Number of plants	Green weight			Air-dry weight		
		Top	Root	Total	Top	Root	Total
Aerated:							
Boron added	3	91.7	50.0	141.7	4.2	0.7	4.9
No boron added	4	62.5	25.0	87.5	3.2	.3	3.5
Unaerated:							
Boron added	6	45.8	16.7	62.5	2.9	.6	3.5
No boron added	6	41.7	20.8	62.5	1.8	.2	2.0

Plants of series 2 were put into nutrient solutions February 25, 1928. When examined on March 12, none exhibited recognizable symptoms of boron deficiency, but on March 14, 8 of the 16 plants in the aerated boron-deficient solutions showed definite signs of injury. At that time only 1 of the 24 plants in the unaerated boron-deficient solution showed symptoms of the trouble. By March 28, 14 of the 16 plants in the boron-deficient aerated solutions showed typical boron-deficiency injury and the 2 plants that did not show the trouble had made little or no growth because of poor root systems at that time. Only 6 of the 24 plants in the unaerated boron-deficient solutions showed boron-deficiency symptoms. Six plants from the aerated and nine from the unaerated boron-deficient solutions were then placed in the solutions containing boron. Like numbers of plants were removed from the solutions containing boron to those deficient in boron. Five plants in the aerated group, which had been in the boron-deficient solutions, and three in the unaerated group exhibited typical boron-deficiency symptoms. The groups of plants changed were of approximately the same size as those remaining in the respective solutions, except the group removed from the solution containing boron to that deficient in boron of the unaerated group. These plants were larger than other plants of this group, as is shown by the measurements given in Table 2.

TABLE 2.—*Average measurements of tobacco plants grown in aerated and unaerated nutrient solutions, with and without the addition of boron, from February 25 to April 11, 1928 (series 2)*

[Amount of boron=0.5 p. p. m.]

Treatment	Number of plants	Height of stalk (cm.)		Length of root (cm.)		Number of leaves		Leaf area (sq. cm.), Apr. 11	
		Mar. 28	Apr. 11	Mar. 28	Apr. 11	Mar. 28	Apr. 11	Total	Per leaf
Aerated:									
Boron added	10	37.7	90.6	34.9	41.0	12.5	18.4	2,045.5	111.1
No boron added	10	16.5	16.9	32.5	35.5	10.9	8.7	1,718.0	197.5
Boron supplied until Mar. 28; then no boron supplied until Apr. 11	6	35.8	71.7	34.3	36.3	12.2	17.7	1,896.5	107.1
No boron supplied until Mar. 28; then boron supplied until Apr. 11	6	13.7	22.3	26.3	32.0	9.5	12.0	1,226.2	102.1
Unaerated:									
Boron added	15	18.1	38.1	7.1	12.5	8.7	11.3	1,055.7	102.2
No boron added	15	19.4	24.0	10.1	13.7	8.9	9.0	1,022.8	113.6
Boron supplied until Mar. 28; then no boron supplied until Apr. 11	9	23.7	51.1	8.7	11.7	9.9	12.8	1,798.9	140.5
No boron supplied until Mar. 28; then boron supplied until Apr. 11	9	19.6	29.8	7.7	11.8	9.4	10.3	1,011.9	98.2

When measured on April 11, the plants grown in the boron-deficient solutions for the whole period had increased only slightly in height and had not developed additional leaves. Those in the solutions containing boron for the entire period, however, had doubled in height and had increased their number of leaves by one-half. The marked effect of aeration on the length of the roots is shown in Table 2. The boron treatment did not greatly affect the total lengths of roots, but a different type of root growth that will be discussed later was produced in the boron-deficient solutions. The average condition of the cultures at this time is illustrated in Figure 2.

The effect of boron on the total leaf area was not so marked as might have been expected, but the average area per individual leaf was greater for plants in both the aerated and the unaerated boron-deficient solutions. The leaves on an average were broader. Thus it appears that boron deficiency produced an effect on leaf size and shape similar to that commonly produced by topping in cultural practice.

The plants that exhibited boron-deficiency symptoms on March 28 when transferred to the nutrient solutions containing boron showed recovery by April 11 and were putting out healthy lateral branches (suckers) in both the aerated (fig. 3) and the unaerated (fig. 4) cultures. Those plants that were transferred from solutions containing boron to those deficient in this element did not show on April 11 any symptoms of boron deficiency in the unaerated group. With the same treatment in the aerated cultures five out of six plants had not produced open flowers; that is, four of this group dropped their buds before the flowers opened. One plant produced open flowers and set one seed pod, but it was further advanced than the others when changed to the boron-deficient solution. One plant did not show any response except delayed growth. Plants in the boron-deficient solutions for the entire period showed no signs of flower stalks or buds on April 11. On the other hand, in the aerated group with boron supplied, all plants had open blossoms or buds almost ready to open. In the unaerated solutions with boron sup-

plied many of the plants showed no signs of buds or blossoms, while others showed well-developed flower buds. The plants in the unaerated solutions deficient in boron showed no flower buds. All plants in the unaerated cultures were much more variable in their responses than those in the aerated cultures. This no doubt was due to poor root development. Most of the plants in these experiments developed considerable mosaic infection just prior to harvest, but as late infection does not materially decrease the growth, no serious consequences followed.

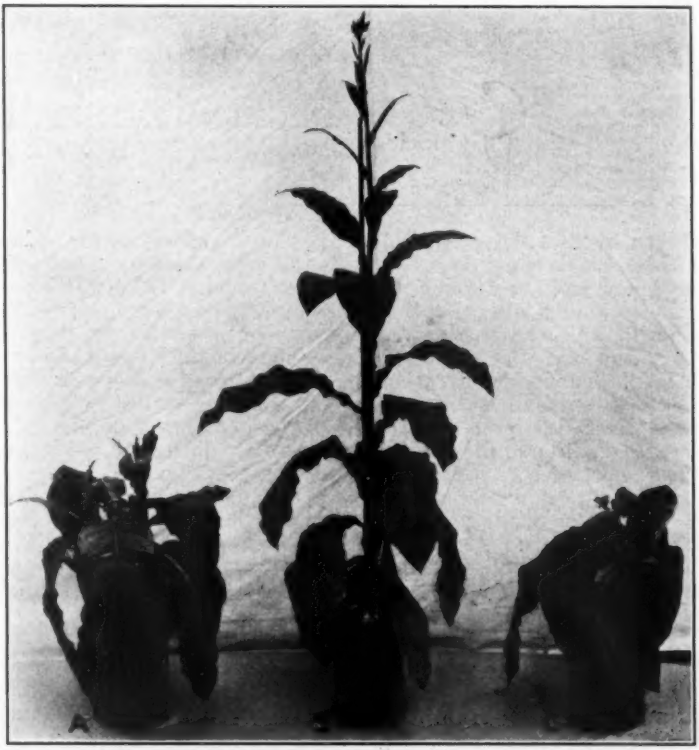

FIGURE 3.—Tobacco plants placed in aerated solutions February 25, 1928, series 2 (photographed April 11, 1928): A, No boron added until March 28—note recovery; B, boron added February 25—note normal plant; C, no boron added during the experiment—note injury

The green and oven-dry weights from series 2 are shown in Table 3. Here again it is evident that on the whole the most marked effect of boron was in stalk development. The final average growth of these cultures is well shown in Figure 2. Here also is apparent the marked effect of aeration on root development, which, in turn, produced decided increase in top growth when boron was added. The total average dry weight of the plants in the aerated cultures with boron supplied is markedly superior to that of the other combinations.

DESCRIPTION OF INJURIES DUE TO BORON DEFICIENCY

It is evident that the total growth of the tobacco plant is reduced when the plant is grown in boron-deficient media, as is shown by the data presented in the tables and by the illustrations. The most characteristic effects, however, are the injuries produced on the growing plant. (Fig. 5.) When once observed they are so characteristic as to be easily identified again. The first distinct symptom of injury is seen in the terminal bud of the vigorously growing plant. The young leaves that make up the bud take on an abnormal appearance, characterized at first by a light-green color at the base. This is followed by the death of the leaf tissues at the bases of the younger leaves and the death and drying of the terminal growing point. The plant subsequently takes on a glabrous appearance. The upper leaves become stiff, brittle, and slightly chlorotic. Such plants were observed to emit a faint aromatic odor with some resemblance to that of the tobacco blossom.

TABLE 3.—*Average green and oven-dry weights (grams) of tobacco plants grown in aerated and unaerated nutrient solutions, with and without the addition of boron, from February 25 to April 12, 1928 (series 2)*

[Amount of boron=0.5 p. p. m.]

Treatment	Number of plants	Green weight				Oven-dry weight			
		Leaves	Stalk	Roots	Total	Leaves	Stalk	Roots	Total
Aerated:									
Boron added	10	60.8	65.1	22.7	148.6	7.1	7.8	1.8	16.7
No boron added	10	59.3	19.9	12.7	91.9	5.6	2.3	1.1	9.0
Boron supplied until Mar. 28; then no boron supplied until Apr. 12	6	55.7	50.7	17.0	123.4	6.6	6.3	1.5	14.4
No boron supplied until Mar. 28; then boron supplied until Apr. 12	6	55.7	32.8	16.9	105.4	5.6	3.6	1.4	10.6
Unaerated:									
Boron added	15	29.6	20.9	10.5	61.0	3.1	2.3	.9	6.3
No boron added	15	26.7	12.8	4.0	43.5	2.9	1.4	.6	4.9
Boron supplied until Mar. 28; then no boron supplied until Apr. 12	9	50.0	36.0	6.9	92.9	5.0	3.9	1.4	10.3
No boron supplied until Mar. 28; then boron supplied until Apr. 12	9	30.2	17.1	6.6	53.9	3.2	2.0	.8	6.0

A plant suffering from boron deficiency will frequently generate a new growing point or points by forming branches (suckers) in the axils of the leaves. These, however, die like the terminal growing point. In some cases buds were observed beneath the cork supports on the roots of plants in boron-deficient solutions. The death of the growing point naturally prevents development of a stalk and, therefore, subsequent flowering. The plants do not die immediately; in fact all remained alive during the periods of observation. In one instance two plants were grown in a boron-deficient nutrient solution from January 7 to May 7, when they were discarded. Although unhealthy in appearance and lacking a normal green color, they continued to live. Because of the inhibiting condition previously described, they were not able to flower. The controls in the solutions containing boron flowered and set seed abundantly.

It is difficult to draw any conclusions from the poor growth of the roots in the unaerated solutions. The roots were short and stubby and their growth was confined to the top of the jar. However, plants grown in the aerated solutions deficient in boron showed roots that differed from those of plants in the solutions containing boron in being brown instead of white. The roots of plants suffering from boron deficiency likewise had many short stubby laterals which are not characteristic of plants grown in solutions to which boron was supplied.

Symptoms of boron deficiency on the tobacco plant can be distinguished from those of calcium deficiency by the fact that the injuries originate in different regions on the young leaves. As previously stated, boron deficiency causes injury at the bases of the young leaves, and the tips remain alive for some time. In some cases, if the injury is not too severe, the leaf will even make some

FIGURE 4.—Tobacco plants placed in unaerated solutions February 25, 1928, series 2 (photographed April 11, 1928; scale in inches): A, No boron added until March 28—note recovery; B, boron added February 25—note normal plant; C, no boron added during the experiment—note injury

growth, but at its base there will be areas of injury that distort its growth. When tobacco is grown in media deficient in calcium its terminal bud is injured, but the typical injuries of the young leaves occur on the margins and tips. Leaves injured when small may later make considerable growth, but the tips and margins will be abnormal in shape. These two deficiencies have, however, symptoms in common in acute cases; namely, that of producing death of the terminal bud or growing point on the tobacco plant.

SUMMARY

It has been found impossible to grow normal tobacco plants to maturity in nutrient solutions containing only the commonly accepted essential elements dissolved in distilled water. It was possible, however, to grow these plants when tap water was used instead of

distilled water. In experiments herein reported, normal tobacco plants were produced when boron (0.5 p. p. m.) was supplied to the solutions made up with distilled water. Growth more especially that of the stalk, was markedly reduced when this element was absent. In connection with these experiments, observations were made on the effect of aeration of the culture medium on the growth of the tobacco plant.

Although growth is greatly reduced in boron-deficient media, the most striking characteristic is the injury of the terminal bud.

FIGURE 5.—Tobacco plant showing characteristic symptoms of boron deficiency. Scale in inches

This injury is more pronounced in vigorously growing plants than in those grown under unfavorable conditions. Thus, plants grown in aerated water cultures developed more pronounced symptoms than those in unaerated cultures, because their growth was more rapid.

Injury from boron deficiency is of such a nature that it is readily distinguishable from a somewhat similar injury due to calcium deficiency. The injury resulting from boron deficiency occurs at the bases of the young leaves that make up the terminal bud, while the injury resulting from calcium deficiency occurs at the tips and margins of the young leaves.

Considerable experimental evidence is presented showing that tobacco plants grow much better in aerated than in unaerated solutions. The most marked effect of aeration is superior root development during the early growth of the plant, followed later by a decided increase in top growth. In preliminary trials no increase in growth was obtained with cultures through which nitrogen gas was bubbled, indicating that the action of the aeration was not due to simple stirring or agitation of the culture media.

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DEVELOPMENT OF THE COTTON BOLL AS AFFECTED BY REMOVAL OF THE INVOLUCRE[1]

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INTRODUCTION

The 3-bracted involucre which subtends the flower in all species of Gossypium (figs. 1 and 2) and which persists until the boll matures is a source of considerable loss in cotton production. The bracts become very dry and brittle by harvest time, and as they envelop the boll rather closely (fig. 3) it is almost impossible to pick the seed cotton from the open bolls without getting fragments of the bracts entangled with the mass of lint hairs. Cotton is sold on grades based largely upon the proportion of foreign material or trash present, and the small pieces of bracts are the largest element of the trash. (Fig. 4.) Part of this trash is eliminated in the cleanings to which the seed cotton ordinarily is subjected before ginning, but much of it is incorporated with the baled fiber and diminishes correspondingly the price received by the grower. Expensive operations in the spinning mills are required to remove the remaining trash.

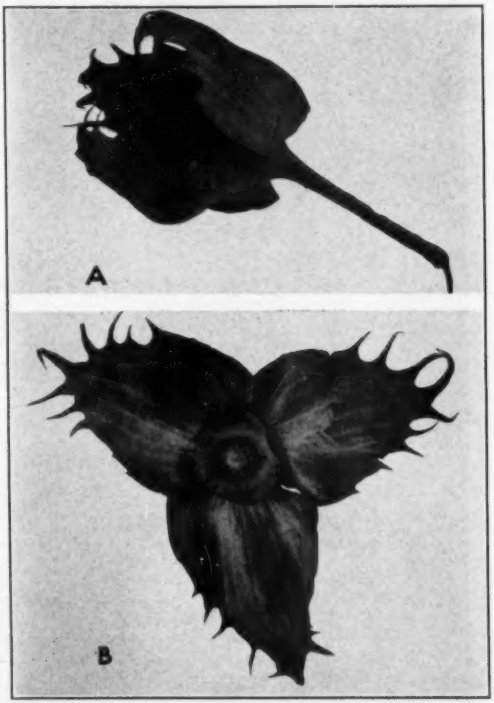

FIGURE 1.—Involucres of cotton of the Egyptian type (Pima variety): A, The involucre in its natural position enveloping a nearly mature boll; B, the involucre and calyx after removal of the boll

Unless the involucre is of physiological importance to the plant, its reduction or suppression would add greatly to the commercial value of cultivated cottons. Complete suppression of this organ

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probably is unattainable, but Cook and Hubbard [2] recently have pointed out the existence in northern South America of a cotton

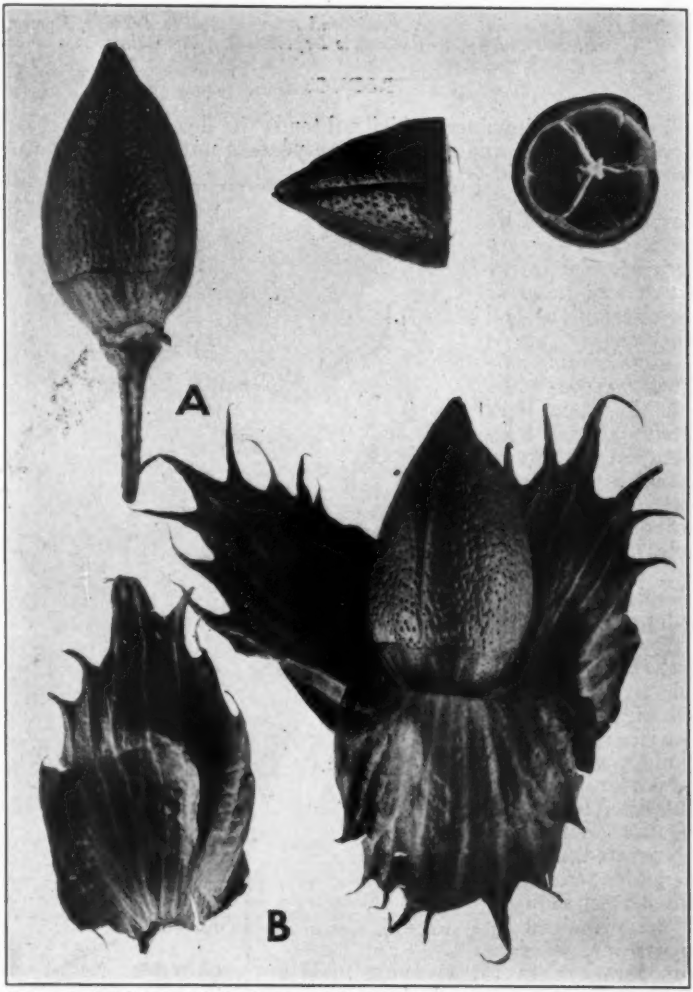

FIGURE 2.—Involucre and bolls of cotton of the Egyptian type (Yuma variety): A, Boll after removal of the involucre; B, illustrating the large size of the involucre relative to that of the boll. The inner structure closely enveloping the lower part of the boll is the calyx, which persists until the boll matures

(*Gossypium evertum* Cook and Hubbard) in which the bracts "stand out separately instead of approaching or forming an inclosure around

[2] COOK, O. F., and HUBBARD, J. W. NEW SPECIES OF COTTON FROM COLOMBIA AND ECUADOR. Jour. Wash. Acad. Sci. 16: 545–552. 1926.
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the bud." Another form (*G. tridens* Cook and Hubbard) is described in the second paper cited as having "very small bracts, narrow and

FIGURE 3.—Ripe bolls of cotton of the Egyptian type, showing portions of the dry and brittle involucre adhering to the masses of seed cotton. Much of the "trash," the presence of which lowers the commercial grade of cotton, consists of fragments of the involucral bracts

separate. The bracts have an abrupt curvature near the base so that they stand out from the bud or boll." Commenting on these

peculiarities, Cook and Hubbard write: "It would be an advantage in a commercial cotton to have the involucres turned back from the bolls, since the fiber undoubtedly could be harvested with less admixture of 'trash,' which consists principally of broken bract material."

The possibility of developing similar characteristics by selection within existing commercial varieties or by crossing them with these South American forms is considered by Cook and Hubbard. They state further: "It is probable that under ordinary cultural conditions the involucre is unnecessary. Buds that have their involucres eaten

FIGURE 4.—Uncleaned seed cotton (Pima variety) as it comes to the gin, showing the fragments of involucral bracts which constitute most of the trash

off by caterpillars or cut off experimentally are able to develop normally into flowers and bolls."

REMOVAL OF THE INVOLUCRE AT ANTHESIS

EXPERIMENT OF 1921

An answer to the question whether or not the involucres are of physiological importance to the plant was sought in an experiment conducted at the United States Field Station, Sacaton, Ariz., in 1921. On each of 50 well-grown plants of Pima cotton, one flower on each of four fruiting branches was treated on the day of anthesis by cutting off the involucre close to the pedicel, while one flower on each of four

branches alternating with these was left unmutilated as a control and was merely tagged. The control flowers were designated A, and the flowers from which the involucre was removed were designated B. A total of 200 flowers received each treatment, but 3 of the control flowers were accidentally destroyed, and these were subtracted from the total in computing the percentage of bolls shed.

When the bolls were thought to have reached full development, but before they opened, their external length and greatest diameter were measured. When they had opened naturally, they were gathered and ginned separately, and the number of seeds and the total weight of seeds in each boll were determined. From these values the mean weight per 50 seeds was computed. A number of the tagged bolls had opened before the external measurements were made, consequently the length and diameter were determined on smaller numbers of bolls than in the case of the determinations of seed weight and number of seeds.

On 25 of the plants a record of the date of anthesis and the date of opening of the boll was kept for each of the A and B bolls, so that the number of days required for the maturation of the boll could be computed. Since this record was kept on only half of the plants, the numbers involved are smaller than for the other characters.

Owing to losses from shedding and from accidental destruction of flowers and of bolls, the several determinations could not be made on all four bolls of each treatment on every plant. In order to make the comparison as fair as possible, all differences were taken by pairs of bolls on the same plant. Thus, if a plant retained only two bolls of treatment A, only two bolls of treatment B on the same plant were used for comparison; and, in order to avoid any selection of the bolls compared, the differences were taken between bolls of the two treatments on the individual plant which had received the same number at the time the flowers were tagged, boll A–2 being compared with boll B–2, for example.

The mean differences, computed in this manner from differences between pairs of bolls, are shown in Table 1. The control flowers (A) gave a higher mean for all characters than the treated flowers (B), although, in respect to the percentage of bolls retained, the difference is not significant, being only 1.5 times its probable error. For all other characters the differences appear to be significant. Expressing the mean difference as a percentage of the mean value for the control (A) bolls, it was found that the removal of the involucre at the time of flowering resulted in an average shortening of the maturation period by about 4.5 per cent and in an average diminution of the external dimensions of the boll, of the number of seeds contained therein, and of the mean weight of the seeds, ranging from 4 to about 9 per cent of the mean value for the bolls from the unmutilated (A) flowers.

It is conceivable that if all the involucres on a plant were removed, the diminution of assimilating tissue might cause a heavier loss than is indicated by the presumably localized effect of removing only four involucres on each plant, as was done in the experiment of 1921. The total surface of the involucres relative to that of the leaves is surprisingly large. Measurements on well-grown plants made under the direction of the F. M. Eaton, at Escondido, Calif., in 1925, showed the ratio of the total area of involucres to the total area of leaves to

be approximately 1:4 in Pima cotton (Egyptian type) and 1:3 in Acala cotton (upland type). If materials elaborated in the chlorenchyma of the involucres contribute to the general nutrition of the plant, and not merely to that of the individual bolls which they subtend, the decrease in production of seeds and lint resulting from the removal of all involucres on the plant probably would be greater than is indicated by the results of this experiment.

TABLE 1.—*Differences between bolls of Pima cotton from unmutilated flowers (A) and from flowers from which the involucres had been removed on the day of anthesis (B), experiment of 1921*

[All differences are taken A minus B, and higher values for the unmutilated flowers are shown in all cases]

Character compared	Number of bolls of each treatment compared	Mean difference [a]	D/E	Difference as related to the mean of A bolls (per cent)
Bolls retained.....per cent	.....	3.30±2.27	1.5	3.7
Maturation period.....days	78	3.15± .37	8.5	4.7
Length.....millimeters	126	3.47± .24	14.5	7.4
Diameter.....do	126	1.11± .12	9.2	4.0
Number of seeds per boll	151	1.01± .23	4.4	5.5
Weight per 50 seeds.....grams	151	.84± .06	14.0	8.8

[a] The probable errors of the mean differences were computed from the array of differences, except that in the case of the percentage of bolls retained the usual formula for the probable error of a percentage was used. The numbers on which the percentages of bolls retained are based were 197 flowers and 175 bolls of the control set (A) and 200 flowers and 171 bolls of the treated set (B). The proportions of bolls actually retained were 88.8±1.5 per cent for the A flowers and 85.5±1.7 per cent for the B flowers.

EXPERIMENT OF 1928

In order to test this possibility a second experiment was conducted at Sacaton in 1928. Ten pairs of well-grown and fertile plants of an inbred and very uniform family of Pima cotton were selected. The plants of each pair were adjacent in the row. On one plant of each pair (B) the involucre of every flower which opened during the period July 20 to August 31 was removed with scissors on the day of anthesis, and a tag bearing the date of flowering was attached. On the control (A) plant of each pair the involucres were left intact, and the flowers were merely marked with dated tags. Flowers on the vegetative branches as well as on the main stalk were treated and tagged on the B plants and were only tagged on the A plants, but the number of flowers borne on the main stalk during the period of the experiment was so large that the bolls borne on the vegetative branches were disregarded in working up the results. The average number of flowers per plant produced on the main stalk in the 42 days during which the experiment continued was 49 for the B plants and 47 for the A plants.[3]

As the bolls opened during the fall, they were harvested daily, each with its tag, and the date of opening of the boll was recorded. The dates of anthesis and opening of the boll being known, it was easy to compute the number of days from flowering to the opening of the boll. This determination was made separately for each tagged

[3] The number of flowers on the individual plants ranged from 34 to 65. The fertility of these plants is shown further by the fact that previous to July 20, the day when the experiment began, they had produced on the fruiting branches of the main stalk an average of 22 bolls per plant.

boll, and the mean for each plant was then computed. In computing, for each plant, the percentage of bolls shed from tagged flowers, all missing bolls were considered as having been lost by natural abscission, although it is probable that a few had been destroyed accidentally. The plants suffered so little damage, however, that the resulting errors in the shedding percentages were undoubtedly negligible.

When the contents of the tagged bolls had dried out thoroughly, determinations of the weight of seed cotton and the number of seeds were made for each boll, separately. The statistical constants of these two characters, for each of the 20 plants, were computed from the determinations on the individual bolls, of which the number per plant averaged 40 for the A plants and 39 for the B plants.[4]

The mass of seed cotton from each plant then was ginned as a unit, and the weight of lint and the number of seeds were determined. From these data the lint index (weight of lint per 100 seeds) was computed for each plant. The seeds from each plant were counted in lots of 50, and each lot was weighed separately. From the resulting data the mean weight of 50 seeds was computed for each plant. The number of lots of 50 seeds varied from 7 to 18 for the individual plants.

TABLE 2.—*Effect of removing the involucre on the day of anthesis: Differences between the means of 10 pairs of plants of Pima cotton, experiment of 1928*

[The B plant of each pair had the involucres of all tagged flowers removed, but on the control plant (A) the involucres were left intact. In each case the difference is taken A minus B, the minus sign therefore indicating that the treated plant of the pair gave the higher value]

Pair No.	Differences in—					
	Percentage of bolls retained	Time from anthesis to opening of boll (days)	Weight of seed cotton per boll (grams)	Number of seeds per boll	Weight of 50 seeds (grams)	Lint index (grams)
1	2.9	7.2	0.80	1.3	0.95	1.59
2	3.6	6.1	.55	.4	1.05	1.11
3	2.4	4.5	.59	.3	.95	1.20
4	−1.3	5.0	.32	0	.60	.94
5	10.7	3.9	.29	−1.1	.99	.93
6	1.8	6.4	.17	−.9	.83	.61
7	30.4	5.6	.44	.1	.90	.99
8	10.0	1.8	.48	.6	.84	.80
9	2.6	3.0	.57	.8	1.08	1.07
10	7.0	3.8	.93	2.4	.98	1.28
Mean difference between populations A and B [a]	7.0±2.0	4.7±.38	0.51±.05	0.4±.22	0.92±.02	1.05±.06
D/E	3.5	12.4	10.2	1.8	46.0	17.5
Difference as a percentage of the mean of population A	8.0	7.8	16.3	2.3	13.8	21.0

[a] Sum of the plus differences minus the sum of the minus differences divided by 10. The probable errors of the mean differences were computed from the arrays of differences and were increased by Pearson's correction for the standard deviation when the numbers are small.

Table 2 presents the results of these determinations, in the form of differences between the 10 pairs of plants, the differences being taken A minus B, so that a minus sign indicates that the plant on which the involucres were removed gave the higher value for the character in question. At the botton of the table are given the mean differences

[4] The number on the individual plants ranged from 26 to 51.

between the treated and untreated plants (sum of the plus differences minus the sum of the minus differences divided by the number of pairs), the values D/E, indicating the number of times the mean difference exceeds its probable error, and the mean differences taken as percentages of the means of the control (A) population for the character in question.

It is seen that the A plant of every pair gave the higher value for each character with the exceptions that the B plant of one pair gave the higher value for percentage of bolls retained and the B plant of two pairs gave the higher value for number of seeds per boll.

Considering the mean differences, the control (A) plants gave a significantly higher value for percentage of bolls retained, number of days from anthesis to the opening of the boll, weight of seed cotton per boll, seed weight, and lint index. Removal of the involucre had resulted in shortening the maturation period by an average of 4.7 days and had caused a very significant decrease in the weight of seed cotton in the boll, in the weight of the individual seeds, and in the quantity of fiber on the seeds (lint index). Comparing the differences on the basis of percentages of the mean value for the control (A) plants, removal of the involucre is seen to have reduced the weight of the bolls (seed cotton), of the individual seeds, and the lint index about 16, 14, and 21 per cent, respectively. On the other hand, the mean number of seeds per boll was not significantly altered by the mutilation.

COMPARISON OF RESULTS OF TWO EXPERIMENTS, 1921 AND 1928

In the experiment of 1921, the involucre was excised from only four flowers on each plant. In the experiment of 1928 the involucres were removed from all flowers which opened during a period of 42 days, coinciding with the period of most active flowering, the number of flowers so treated having averaged 48 per plant. In the former case an insignificant fraction and in the latter case a considerable proportion of the total chlorophyll-bearing tissue of the plant was removed. It will be of interest to compare the mean differences between the control and the mutilated plants for the characters which were determined in both experiments. This comparison is given in Table 3 and shows no significant differences in the magnitude of the reduction caused by the removal of the involucre in the experiments, except possibly in the number of days from anthesis to the opening of the boll, in which case the difference is three times its probable error.

TABLE 3.—*Mean differences between the control (A) plants and the plants which had the involucres removed at anthesis (B) in the experiments of 1921 and 1928*

Experiment	Differences in—			
	Percentage of bolls retained	Time from anthesis to opening of boll (days)	Number of seeds per boll	Weight of 50 seeds (grams)
1921	3.3±2.27	3.1±0.37	1.0±0.23	0.84±0.06
1928	7.0±2.00	4.7± .38	.4± .22	.92± .02
Difference	3.7±3.02	1.6± .53	.6± .32	.08± .06
D/E	1.2	3.0	1.9	1.3

It would seem, therefore, that the effect of removing the involucre is largely a local one and that whatever contribution this organ makes to the nutrition of the plant is limited to the flower and boll subtended by it. If this were not the case, the experiment of 1928, in which a much larger number of involucres were removed from each plant, should have resulted in a substantially greater reduction in seed weight than occurred in the experiment of 1921.

In both experiments, the effect of removing the involucres upon the number of seeds in the boll was relatively insignificant, and indeed a significant effect upon this character was scarcely to have been anticipated. Since the number of ovules is determined long before anthesis, conditions affecting fertilization alone could be expected to cause a difference in the mean number of seeds, and it is not apparent how removal of the involucre could affect fertilization.

The reduction in weight of the individual seeds in bolls deprived of the involucre is very significant and of practically the same magnitude in both experiments. In 1921 it amounted to 9 per cent and in 1928 it was 14 per cent of the weight of the seeds in the bolls from unmutilated flowers. This fact, together with the reductions, in the experiment of 1928, of 16 per cent in the weight of seed cotton per boll and of 21 per cent in the weight of fiber per 100 seeds (lint index), points strongly to the conclusion that the involucre plays an important part in the nutrition of the boll subtended by it. The possibility of a wound effect is not excluded, of course, although the excision of the involucre was performed with every care to avoid injury to the flower and its pedicel.

The withdrawal of the shade which the involucre affords the young boll is very probably also a factor in the results from excision. In the shortening of the period from anthesis to the opening of the boll, which averaged 3.1 days in 1921 and 4.7 days in 1928, this may well have been the chief factor, since full exposure to the sun and the consequent more rapid drying of the external walls of the carpels doubtless hastens dehiscence. Some of the bolls from which the involucres had been removed showed external evidence of sunburn, and in a few cases this extended to the fiber within, which was discolored as though by the action of fire.

The data of these experiments throw no light on the question whether removal of the involucre altered the length of the period required for the maturation proper rather than the opening of the boll, since the period measured was from anthesis to separation of the carpels and hence included a postmaturation period which doubtless covered several days. It is conceivable, at least, that the increase in the amount of light reaching the unprotected surface of the boll and the resulting increase in its temperature would hasten maturation, aside from any subsequent effect upon the rapidity of dehiscence.

REMOVAL OF THE INVOLUCRE BEFORE ANTHESIS [5]

A second experiment was conducted at Sacaton in 1928 with the object of comparing the effects of removal of the involucre at an earlier stage in the development of the flower with the effects of removal on the day of anthesis.

[5] This experiment was performed by Robert H. Peebles, senior scientific aid, formerly of the Office of Alkali and Drought Resistant Crops, now of the Office of Horticultural Crops and Diseases.

Thirty consecutive plants in a row of Pima cotton were numbered successively A–1, B–1, A–2, B–2, and so on. On the B plants the involucres were removed with a pair of small scissors from flower buds ranging in length from 7 to 15 mm. (average length 10.9 mm.), whereas on the A plants buds of approximately the same size (average length 10.6 mm.) were left unmutilated, as controls. A small tag bearing the date was attached to each bud. The number of tagged buds differed considerably on the individual plants, but the involucres were removed on a total of 170 buds on the B plants, and an equal number of buds were tagged on the control (A) plants.

A record was kept of the date of anthesis of each tagged bud and of the number of tagged buds shed before and after anthesis on each plant. From these data were computed, for each of the 30 plants, the mean number of days to anthesis from the time the bud had reached the average length mentioned and the percentage of buds and of bolls that were shed. The number of days to anthesis averaged 12.8 for the control (A) flowers and 12 days for the mutilated (B) flowers.

TABLE 4.—*Effect of removing the involucre at 10 to 14 days before anthesis: Differences between the means of 15 pairs of plants of Pima cotton, experiment of 1928*

[The B plant of each pair had the involucres of all tagged buds removed, but on the control plant (A) the involucres were left intact. In each case the difference is taken A minus B, the minus sign therefore indicating that the treated plant of the pair gave the higher value]

Pair No.	Differences in—							
	Percentage of buds	Percentage of bolls retained	Time to anthesis (days)	Weight of seed cotton per boll (grams)	Number of seeds per boll	Weight of 50 seeds (grams)	Lint index (grams)	Lint length (millimeters)
1	0	3.2	0.7	0.55	3.0	0.55	0.81	2.7
2	16.9	10.0	1.2	1.42	2.9	1.10	1.48	2.4
3	13.4	14.0	2.3	.26	−.2	.60	.76	1.8
4	8.3	18.2	.6	1.05	1.5	1.65	.70	3.9
5	18.2	7.9	1.6	.78	2.5	.30	.61	−.3
6	0	8.2	1.2	.76	1.9	.85	.77	2.4
7	7.7	1.9	.6	1.23	3.6	.55	1.40	3.3
8	−13.3	−14.1	1.5	.90	.9	1.25	1.11	3.9
9	0	11.4	.5	.10	−.6	.65	.72	2.1
10	15.3	9.8	1.2	.87	2.6	1.20	1.15	4.8
11	0	16.6	.3	.54	.2	1.20	1.08	2.7
12	0	−18.2	1.2	.66	1.5	.75	.59	3.6
13	0	12.2	.4	1.01	1.9	1.15	.86	4.2
14	0	1.8	−.7	.90	3.6	.95	1.19	2.4
15	0	−25.0	−.7	.52	.8	.55	.89	3.6
Mean difference between populations A and B *	4.4±1.5	3.9±2.3	0.8±.14	0.77±.06	1.7±.23	0.89±.08	0.94±.05	2.9±.22
D/E	2.9	1.7	5.7	12.8	7.4	11.1	18.8	13.2
Difference as a percentage of the mean of population A	4.5	4.5	6.2	23.3	9.2	14.1	19.2	7.4

* Sum of the plus differences minus the sum of the minus differences divided by 15. The probable errors of the mean differences were computed from the arrays of differences and were increased by Pearson's correction for the standard deviation when the numbers are small.

The tagged bolls were harvested as they matured, and the weight of the seed cotton and the number of seeds per boll, the weight of 50 seeds, and the lint index were computed in the manner already described for the experiment of 1928 in which the involucres were

removed on the day of anthesis. In addition, the length of the lint was measured on one seed from each boll. From these data the means for these five characters were computed for each plant.

The results are stated in Table 4 in the form of arrays of differences between the 15 pairs of adjacent plants A–1 and B–1, A–2 and B–2, and so on. The difference is taken in all cases as A (control) minus B (treated), so that a minus sign indicates that the higher value was yielded by the plant from which the involucres were removed. Table 4 gives also, in respect to each character, the mean difference between populations A and B, with its probable error computed from the array of differences, the mean difference divided by its probable error (D/E), and the mean difference as a percentage of the actual mean of the control population (A) for the character in question.

A comparison of Table 4 with Table 2, which gives similar data for the other experiment of 1928, shows relatively little difference between the effects of removing the involucre at 10 to 14 days before anthesis and the effects of removing it on the day of anthesis, except in respect to number of seeds per boll. The percentage either of buds or of bolls retained was not significantly affected by the removal of the involucre in the bud stage (Table 4), although the percentage of bolls retained was reduced, apparently significantly, by the removal of the involucre on the day of anthesis (Table 2). The average length of the period to anthesis from the time the bud is about 11 mm. in length was reduced 6 per cent by the removal of the involucre in the bud stage (Table 4), whereas the average length of the period from anthesis to the opening of the boll was reduced 8 per cent by the removal of the involucre on the day of anthesis (Table 2).

The average dry weight of the seed cotton in the ripe boll was reduced 23 per cent by the removal of the involucre in the bud (Table 4) as compared to a reduction of 16 per cent when the removal of the involucre was postponed until the day of anthesis (Table 2). The reduction, relative to the control, in the average weight of 50 seeds and in the average weight of lint per 100 seeds (lint index) was practically the same whether the involucre was removed in the bud stage or at anthesis. Apparently these three characters are much more affected by conditions obtaining after than before anthesis.

The most striking effect of the earlier removal of the involucre is shown in the reduction in the mean number of seeds per boll from mutilated as compared with those from unmutilated flowers. Removal of the involucre in the bud caused a reduction which averaged nearly two seeds per boll and was certainly significant (Table 4), whereas the removal of the involucre on the day of anthesis caused a reduction which averaged less than one-half seed per boll and was not at all significant (Table 2). It was noted on a preceding page that the removal of the involucre at the time of flowering, when the ovules are fully developed, could not be expected to affect the degree of fertilization, hence the number of seeds produced. On the other hand, it is readily understandable that the removal of the involucre at 10 to 14 days before anthesis might bring about a reduction in the number of fertile ovules and consequently in the number of seeds.

The removal of the involucre at 10 to 14 days before anthesis, as shown in Table 4, reduced the average length of the lint, as compared with that of the controls, by nearly 3 mm., and the reduction was

highly significant. The effect upon lint length caused by removing the involucre on the day of anthesis was not determined, but it is probable that this character, like weight of seed cotton, weight of seeds, and lint index, is affected chiefly by conditions prevailing after anthesis.

CONCLUSIONS

The results of the experiments described in this paper point to the conclusion that the involucre of Gossypium plays an important part in the development of the flower and boll which it subtends. Suppression of this organ on plants of Pima cotton (Egyptian type) at the time of anthesis caused a marked reduction in the size and weight of the boll, in the weight of the seeds, and in the abundance of the lint. When the involucre was removed about 12 days before anthesis, the same was true and there was also a reduction in the number of seeds in the boll, presumably due to the undernutrition and resulting sterility of some of the ovules.

It would seem that the involucre contributes little to the general nutrition of the plant, notwithstanding the fact that in Pima cotton the total involucral surface may constitute one-fifth of the total chlorophyll-bearing surface. This is deduced from the fact that when the involucres were removed from all flowers opening during a period of six weeks, the average number having been 48 on each plant, the seeds produced by the treated flowers showed no greater reduction in weight, in comparison with the seeds from unmutilated flowers on the control plants, than occurred when only four involucres on each plant were removed. If carbohydrates synthesized in the chlorophyll tissue of the involucral leaves circulate in the plant as freely as similar material elaborated in the foliage leaves, the elimination of so large a proportion of the chlorophyll tissue would be expected to have a deleterious effect on the plant as a whole and hence cause a greater reduction of the seed weight than resulted from the removal of so insignificant a fraction of the chlorophyll tissue as is represented by only four involucres on each plant.

Assuming, as seems probable, that the slight wounds inflicted in cutting away the involucres were not in large measure responsible for the losses observed, the results of these experiments make it doubtful whether reduction of the involucre would be a desirable objective in breeding work. It is to be feared that what would be gained in cleaner picking due to the absence of fragments of involucres in the seed cotton, would be more than offset by the diminished yield of seed and lint.

It does not necessarily follow, however, from the injurious effect of removing the involucres in a cotton having this organ highly developed, that plants in which the involucre naturally is greatly reduced would be inferior in yielding capacity. Such plants might be expected to possess compensating advantages of one sort or another. In any event, cottons like the South American form described by Cook and Hubbard, in which the involucre is present but spreads away from the boll, would afford clean lint without the loss of whatever contribution this organ makes to the nutrition of the boll.

SUMMARY

Complete removal, by excision, of the involucres of Pima cotton flowers on the day of anthesis caused very significant reduction in the external dimensions of the boll, in the weight of the dry contents of the boll, in the weight of the individual seeds, and in the abundance of the lint (lint index). (Tables 1 and 2.)

The length of the period from anthesis to the opening of the bolls was shortened by 3 to 4½ days on an average. (Table 3.) The more rapid drying of the walls of the boll due to full exposure to the light is believed to have been the chief factor in hastening the opening of the bolls deprived of involucres.

The reduction in the weight of the individual seeds, as shown in Table 3, was virtually the same whether the involucres were removed from only 4 flowers on each plant or from all flowers produced during a period of 6 weeks, an average of 48 flowers per plant. This suggests that the involucre contributes materially to the nutrition of the boll subtended by it, but little, if at all, to the nutrition of the plant in general.

The reduction, relative to the control (unmutilated) flowers, in the weight of seed cotton per boll, in the weight of the individual seeds, and in lint index was substantially the same whether the involucres were removed on the day of anthesis (Table 2) or about 10 to 14 days before anthesis (Table 4). The inference is that these characters are controlled mainly by conditions prevailing after anthesis.

The mean number of seeds per boll was little if at all reduced by the removal of the involucre on the day of anthesis (Table 2), but it was significantly reduced when the involucre was removed 10 to 14 days before anthesis (Table 4).

In the former case the ovules were fully developed and the removal of the involucre could have brought about a reduction in the number of seeds only by affecting fertilization adversely. In the latter case inferior nutrition due to suppression of the involucre may have rendered some of the ovules incapable of fertilization.

The effect upon the length of the lint was not determined in the experiments in which the involucre was excised on the day of anthesis, but excision at 10 to 14 days earlier resulted in reducing the mean length by nearly 3 mm., and the reduction was highly significant. (Table 4.)

EXPERIMENTAL STUDIES OF BOVINE LEUKEMIA [1]

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INTRODUCTION

Leukemia is essentially a blood disease in which there is more or less enlargement of the blood and lymph forming organs. There is usually a very marked increase in the number of white blood corpuscles with a possible decrease in the number of red cells.

Leisering [2] is credited with being the first to demonstrate the presence of leukemia in animals in 1858, but from an investigational standpoint the disease received little attention until a much later date. Nocard, Mosler, Cadiot, Roger, Gilbert, Marek, Stockman, and a number of other European investigators have from time to time made studies of leukemia in animals, but it appears that comparatively little investigational work has been done on the disease in the United States.

The various studies of leukemia in mammals have failed to reveal the etiological factor involved, and all efforts made to transmit the disease from animal to animal, through experimental inoculations or otherwise, have thus far given no definite results.

On the other hand, certain European investigators have been able to transmit chicken leukemia through experimental inoculations, thus establishing the infectious nature of the disease in fowls. The present writers, however, have been unable to reproduce the disease in chickens by experimental inoculations of tissue emulsions from what appeared to be typical cases of avian leukemia.

In dealing with the subject of leukemia it becomes necessary to differentiate between the true leukemia, in which there are very marked numerical changes in the cellular elements of the blood, and the so-called pseudoleukemia, in which the blood and lymph forming organs are similarly affected but without apparent changes in the cell content of the blood.

It appears that leukemia is more rarely encountered, in some species of animals, at least, than pseudoleukemia. According to Ostertag,[3] for example, pseudoleukemia is of more frequent occurrence in cattle and hogs than the true form of the disease.

ORIGIN AND HISTORY OF CASE STUDIED

The affected cow, which was the subject of these studies, originated in the State of Maryland, not very far from the District of Columbia. The attention of the writers was first called to her by the owner, who had observed an abnormal condition in the cow and offered to contribute her to this department for experimental purposes. On clinical

[1] Received for publication Dec. 14, 1928; issued April, 1929.

[2] HUTYRA, F., and MAREK, J. SPECIAL PATHOLOGY AND THERAPEUTICS OF THE DISEASES OF DOMESTIC ANIMALS. Authorized Amer. Ed. 3 from rev. and enl. Ger. ed. 6., v. 3, p. 119. Chicago. 1926.

[3] OSTERTAG, R. HANDBOOK OF MEAT INSPECTION. Authorized translation by E. V. Wilcox. Ed. 2, rev. p. 373. New York. 1905.

examination she was found to be typically leukemic in appearance. Subsequently she was purchased from the owner and brought to the experiment station at Bethesda, Md., for observation and study.

It is of some interest, and possible significance, to refer in this connection to the statement of the owner that another of his cows had been similarly affected about two years previously. The visible enlargement of the superficial lymph glands which was also noted in that case was thought by the attending veterinarian to be due to tuberculosis, but the cow failed to give a positive reaction to the tuberculin test, and, according to his statement, showed no lesions of tuberculosis upon subsequent slaughter.

FIGURE 1.—Leukemic cow, showing greatly enlarged prescapular lymph gland

CLINICAL EXAMINATION OF LEUKEMIC COW

The outstanding deviation from the normal noted on clinical examination of the cow was the very marked enlargement of the prescapular, precrural, and supramammary lymph glands. (Figs. 1 and 2.) The animal also exhibited some evidence of difficult breathing. The latter symptom was found later, on autopsy, to be due to chronic pneumonia. A preliminary blood count confirmed the belief that the case was one of true leukemia.

EXPERIMENTAL OBJECTIVES

Since it was definitely determined that the cow was affected with the true form of leukemia, the opportunity appeared favorable to study any possible etiological factor involved, by cultural methods and otherwise, and to endeavor to reproduce the disease through experimental animal inoculations.

BLOOD COUNTS

A limited number of blood counts were made, at short intervals, with a view to observing any possible change in the cytological ratio during the course of the disease. This study was terminated rather unexpectedly by the sudden crisis in the course of the disease which necessitated the slaughter of the animal. The number of counts made showed comparatively slight variations in the cell ratio with the exception of the last, in which it was found that the ratio of red to white cells had made a precipitous drop.

Table 1 shows the blood counts made on the respective dates. The chamber used in making these determinations, as well as other parts of the counting apparatus, had been approved by the United States Bureau of Standards. On two occasions when blood counts

FIGURE 2.—Another view of the leukemic cow, showing the enlarged prescapular, precrural, and supramammary lymph glands

were being made, determinations were also made of the hemoglobin content of the blood, using Ernst Fleischl's hemoglobinometer.

TABLE 1.—*Blood cell counts, with ratio, and hemoglobin content of blood of leukemic cow*

Date	Number of white cells	Number of red cells	Ratio	Hemoglobinometer reading	Remarks
Apr. 6, 1926	102,400	5,246,800	1 to 51	----------	
Apr. 9, 1926	117,000	6,640,000	1 to 56	* 35	
Apr. 14, 1926	101,875	5,840,000	1 to 57	----------	
Apr. 19, 1926	203,250	5,520,000	1 to 27	* 35	Animal showed great weakness.

* The hemoglobinometer reading of a normal cow's blood, using the same apparatus and technic, was 60.

About three weeks after the cow arrived at the experiment station she was reported unable to stand even after being assisted to her feet. Besides the extreme weakness she also showed difficult respiration. Under the circumstances it was thought best to slaughter her immediately.

AUTOPSY FINDINGS

On bleeding the animal it was noted that the blood was pale in color and did not coagulate very readily. The mucous and serous membranes were very pale. There was evidence of emaciation.

The heart was paler than normal and showed numerous small whitish areas scattered through the musculature.

The lungs exhibited a well-defined chronic pneumonia, which accounted for the difficult breathing noted for some time prior to slaughter. The bronchial and mediastinal glands were enlarged to several times their normal size.

The liver was considerably enlarged, with thickened borders, and was rather light in color.

The spleen exhibited the usual leukemic appearance. The surface was uneven or slightly nodulated. The capsule was thickened and the cut surface showed thickly distributed whitish nodules (enlarged follicles) throughout the splenic pulp, which was rather dark or brownish red in color. On account of the very marked enlargement of the spleen and certain of the lymphatic glands these were measured and weighed. The spleen was found to be 10 inches wide at the middle and 26½ inches in length. The weight of this organ was 11 pounds and 5 ounces. Sisson [4] gives the normal dimensions of this organ in cattle as 5 to 6 inches wide

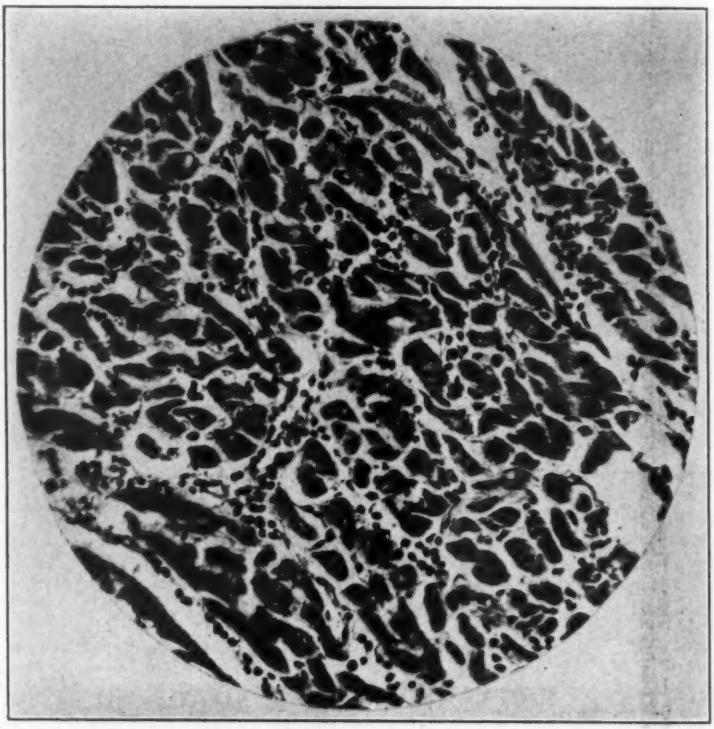

FIGURE 3.—Section of heart of leukemic cow showing lymphocytic infiltration of the musculature. The section also shows evidence of degenerative changes in muscle fibers. Magnified 250 times

and 16 to 20 inches long. He also states that the average weight of the ox spleen is about 30 ounces.

The kidneys were lighter in color than normal, but did not appear to be very much enlarged.

The associated lymphatic glands of the organs described were very noticeably enlarged.

Little change was noted in the digestive tract other than the pronounced increase in the size of the mesenteric lymph glands.

A fetus weighing 8 pounds and 4 ounces was removed from the uterus. Autopsy showed no visible abnormalities either in the principal organs or lymphatic glands of the fetus.

[4] SISSON, S. A TEXTBOOK OF VETERINARY ANATOMY. p. 404, illus. Philadelphia and London. 1910

The prescapular glands of the cow were the most markedly enlarged of the lymphatic glands. One of these glands measured 10 inches in length and 6½ inches in width, and weighed 2 pounds and 10 ounces. Sisson [5] gives the normal dimensions of these glands as 1 or more inches wide and 4 or 5 inches long.

The supramammary lymphatic glands were also greatly enlarged. Paired, these glands measured 10 inches in length and 6½ inches in width. The weight of the two glands was 4 pounds and 10 ounces.

The weight of the precrural glands singly was 14½ ounces.

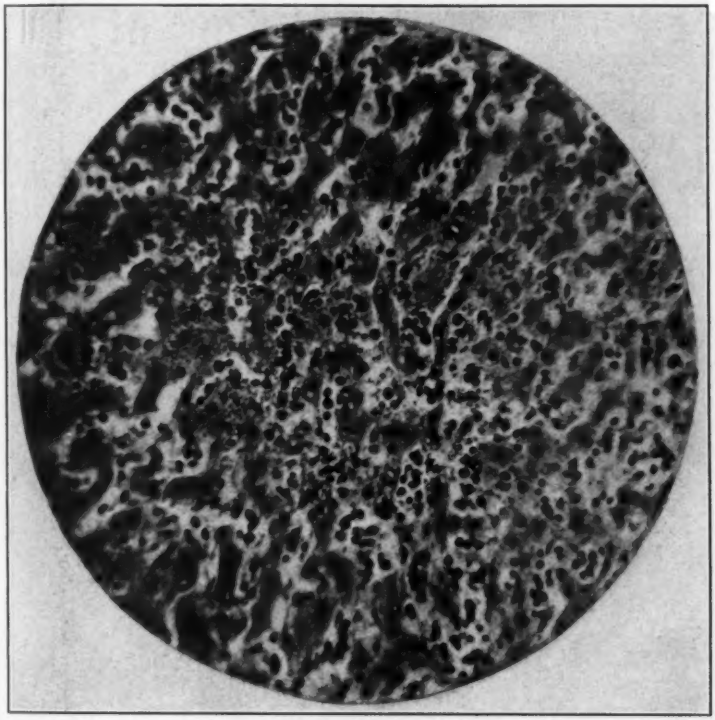

FIGURE 4.—Liver section, showing capillary engorgement and infiltration of white cells between the columns of liver cells. Magnified 250 times

TISSUES CULTURED

The mediastinal, prescapular, precrural, and supramammary lymphatic glands, and also the spleen and liver, were cultured on various media including serum-agar slants, 0.1 per cent agar, and Smith tubes of liver dextrose; the last mentioned for anaerobic microorganisms in particular.

Cultural results were entirely negative.

HISTOPATHOLOGY

HEART.—Sections from the heart muscle showed a limited lymphocytic infiltration, chiefly between the larger bundles of fibers. The musculature stained rather poorly and the fibers showed loss of striations and other evidences of degeneration. (Fig. 3.)

[5] SISSON, S. Op. cit., p. 624, illus.

LUNGS.—There was evidence of a rather extensive lymphocytic invasion of the lungs, in addition to the well-marked catarrhal pneumonia present.

LIVER.—The liver sections showed some capillary engorgement with small hemorrhages in places. There was quite an extensive white-cell invasion of the organ, the cells penetrating not only between the lobules but also between the columns of liver cells. There was also a cellular infiltration of the interstitial tissue. (Fig. 4.) The exces-

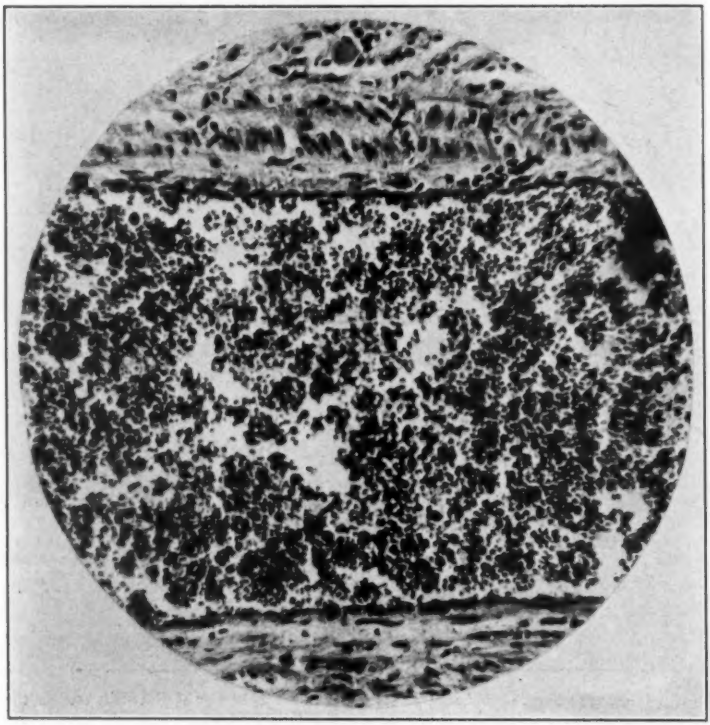

FIGURE 5.—Showing the excessive number of white cells intermixed with the erythrocytes in an artery of the liver. Magnified 250 times

sive number of white cells present in the blood could readily be seen in the larger vessels of the liver sections. (Fig. 5.)

SPLEEN.—The changes in the histologic appearance of the spleen were quite marked. There was a red-cell engorgement in addition to the large masses of white cells invading the splenic pulp. Most of the Malpighian corpuscles were completely obliterated by the lymphocytic infiltration. The trabeculae had largely disappeared, only portions being visible here and there. The capsule of the organ showed some cellular infiltration and was somewhat thickened. (Fig. 6.)

LYMPH GLANDS.—All the noticeably affected lymph glands presented practically the same histological picture. The accumulation of lymphocytes in the glands resulted in complete distortion of the normal structure. The lymph cords and sinuses were crowded with masses of the lymphocytes, and the germinal centers could no longer be recognized.

The capsules and trabeculae were thoroughly infiltrated and in some sections only traces of the latter could be seen. Some of the

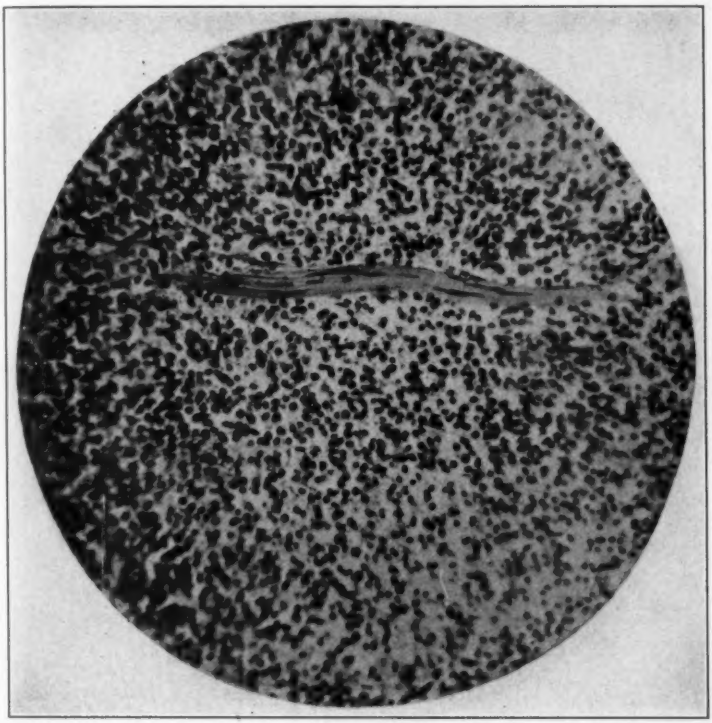

FIGURE 6.—Section of spleen, showing lymphocytic engorgement of the organ. A small remaining portion of one of the trabeculae is seen in the center of the field. Magnified 250 times

glands showed capillary engorgement which in places resulted in small hemorrhages. (Figs. 7 and 8.)

ANIMAL INOCULATIONS

A number of preliminary inoculations of small animals were made shortly after the cow was received at the experiment station. Four guinea pigs were inoculated intraperitoneally and two rabbits intravenously with varying amounts of blood obtained from the jugular vein of the leukemic cow.

The guinea pigs either died or were slaughtered at periods ranging from one month to more than a year. None of these showed any

lesions indicative of leukemic disease. The two rabbits, likewise, showed no evidence of leukemia on autopsy approximately one year after inoculation.

Just prior to the time of slaughter of the leukemic cow, blood was drawn from the jugular vein and another cow was inoculated immediately with 10 cubic centimeters of the warm blood, intravenously. A weanling calf and one sheep were also inoculated intravenously with 5 cubic centimeters each of the cow's blood.

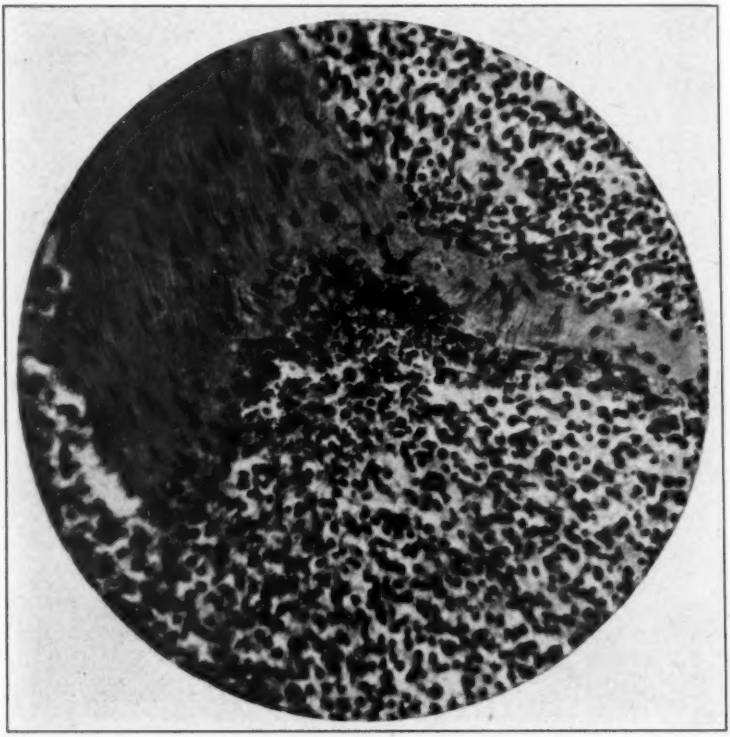

FIGURE 7.—Section of one of the enlarged lymph glands, showing the lymphocytic engorgement of the gland and the cell encroachment on the trabecula, a considerable portion of which has disappeared. Magnified 250 times.

The autopsy findings on the experimental sheep, 10 months after inoculation, were negative for leukemia. The experimental cow and calf were slaughtered more than a year after inoculation and neither exhibited any lesions suggestive of leukemia.

Four guinea pigs were inoculated intraperitoneally and two rabbits intravenously with composite samples of emulsion of the enlarged lymph glands of the cow. The guinea pigs and rabbits either died or were slaughtered at periods varying from approximately one month to more than one year. None of these animals showed any evidence of leukemia on autopsy.

During the year that the experimental cow was held under observation a number of blood counts were made in order to note any possible numerical deviation from the normal of the cell content during this period which might give evidence of a leukemic condition. That no such changes occurred in the blood is indicated in Table 2.

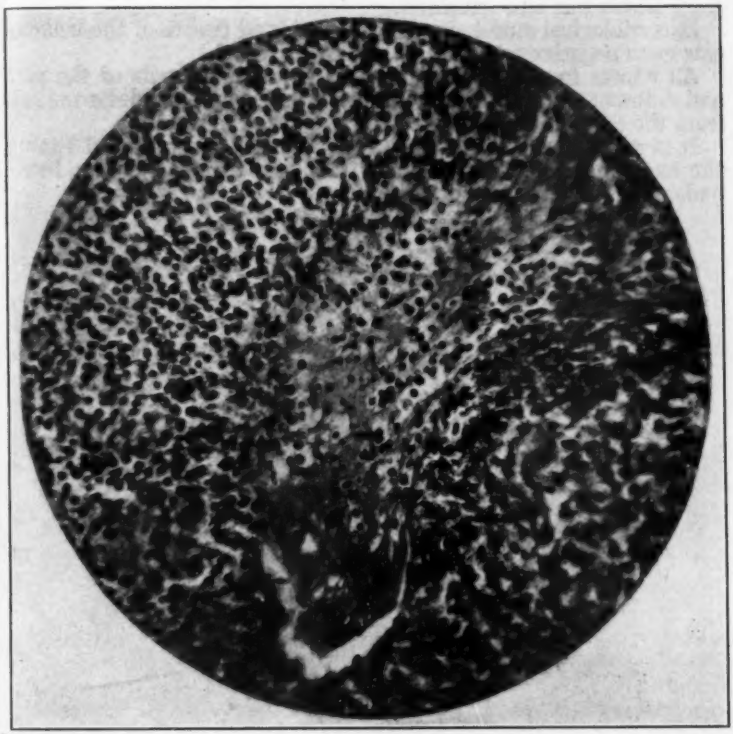

FIGURE 8.—Section of lymph gland, showing excessive accumulation of lymphocytes and also cellular infiltration of a trabecula. The histological picture is typical of the various glands affected. Magnified 250 times

TABLE 2.—*Blood cell counts, with ratio, of blood from experimental cow intravenously inoculated with warm blood from the leukemic animal*

Date	Number of white cells	Number of of red cells	Ratio
Apr. 24, 1926 *	15,750	7,160,000	1 to 454
July 21, 1926	11,625	7,100,000	1 to 610
Oct. 5, 1926	13,750	6,840,000	1 to 497
Dec. 22, 1926	14,320	6,480,000	1 to 452
Mar. 9, 1927	11,250	5,900,000	1 to 524

* Preliminary count made on day cow was inoculated.

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SUMMARY AND CONCLUSIONS

The very marked and definite change in the numerical ratio of the blood cells, as demonstrated by a number of cell counts, together with the typically leukemic changes noted histologically, are considered sufficient evidence that the disease of the cow studied by the authors was true leukemia.

Bacteriological studies made of the affected tissues of the leukemic cow gave negative results.

All efforts to transmit the disease to other animals of the same and different species, by inoculations of blood and glandular material from the affected animal, likewise gave negative results.

It is concluded, therefore, that no evidence was obtained through the experimental studies made of this case to indicate that bovine leukemia is of infectious origin.

RELATIVE PRODUCTIVITY OF SEED POTATOES GROWN UNDER VARIOUS CONTROLLED ENVIRONMENTAL CONDITIONS[1]

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INTRODUCTION

Much has been said and written concerning the supposed effects of various environmental conditions upon the productiveness of seed potatoes. The superiority of northern grown seed potatoes is generally recognized by the commercial industry and arguments pro and con are advanced concerning the importance of soil texture, soil moisture, temperature, etc., in their production. Strains of potatoes deteriorate with greatly varying degrees of rapidity under different environmental conditions. In the earlier research such differences were commonly attributed directly to the environmental conditions. Research during the last 10 years has indicated, however, that the several virus diseases of potatoes are also very responsive to environmental conditions, and that the more or less rapid "running out" of lots of seed potatoes is due to the presence and rapid spread of one or more of these diseases.

DEFINITION OF THE PROBLEM

In view of these facts it seemed desirable to determine whether (or to what extent) the various environmental factors exert an influence upon the productivity of the seed potatoes produced when virus diseases are eliminated. The environmental factors selected for investigation were soil temperature, soil moisture, and soil texture.

EXPERIMENTAL METHODS

Potatoes of the Triumph variety produced on dry land in western Nebraska were planted in the greenhouse in the middle of October and the crop was harvested in February or March. The crop thus grown constituted the seed potatoes which were planted in field plots later in the spring. These seed potato crops were produced five successive years, commencing with 1922.

The normal resting period of the tubers was broken by scraping away the periderm around the eyes, as recommended by Appleman.[2] Seed pieces from each potato were distributed for planting under each set of treatments in the experiment concerned, apical and basal end pieces being apportioned equally among the several treatments.

An air temperature of between 65° and 70° F. was maintained in the greenhouse by thermostatic control. The greenhouse was frequently fumigated with cyanide gas to control aphids, white flies, etc.

[1] Received for publication Nov. 26, 1928; issued April, 1929. Published with the approval of the director as paper No. 66, Journal Series, Nebraska Agricultural Experiment Station.

[2] APPLEMAN, C. O. BIOCHEMICAL AND PHYSIOLOGICAL STUDY OF THE REST PERIOD IN THE TUBERS OF SOLANUM TUBEROSUM. Md. Agr. Expt. Sta. Bul. 183: 200. 1914.

Earthen jars of 4-gallon capacity or galvanized iron cans 2 feet deep and 1 foot in diameter were used for most of the experiments.

The soil was mixed so as to contain organic matter and sand in sufficient quantity for good greenhouse culture, except where soil texture was the factor studied.

To prevent soil cracking, rapid evaporation, etc., a 2-inch layer of gravel was placed on top of the soil. In the case of the soil-temperature series this was found inadequate because the tubers developed in the gravel where they were not completely under the desired soil-temperature conditions. This difficulty was eliminated after the first year by substituting mineral wool for gravel and then placing a circular piece of sheet iron (with a hole in the center for plant emergence) between the mineral wool and the soil so as to prevent tuber formation in the insulation material.

In the soil-temperature series, the air temperature was the same as for the other experiments, but the soil temperature was varied by sinking cylindrical cans of soil (24 by 10 inches) into insulated tanks of water maintained at different temperatures. The desired temperatures were secured by steam and cold-water connections. By checking two or three times a day, such temperatures were maintained that the extreme variations of two adjacent series did not overlap. Temperatures were recorded either by periodic readings or by thermographs.

Soil moisture was modified in one series by maintaining different gross weights, which were checked by weighing at regular intervals. The maximum amount of water that the soil would retain against gravity (as determined in one of the containers) was used as the basis for determining the amount of water to be used. The water was added on to the surface of pots in part of the series and through a tube into a reservoir made by inverting a clay pot in the center of the potometer in the other pots of the series. One year the surface and interior systems of applying water were compared. As no difference was noted in any of the results this test was discontinued. In the case of the series designated as 100 per cent a water table was maintained in a layer of gravel in the bottom of a can by connection with a reservoir.

Soil texture and structure were varied by building up synthetic soils, using good surface soil (silt) and river sand, or by the addition of different amounts of composted manure (which would also increase the fertility).

The experimentally produced tubers were harvested in February or March and stored until the following June, when they were planted under field conditions in western Nebraska for comparative trials in replicated plots. The data in this paper are based on these trials. In the tables each "mean" represents the yield from 20 to 50 plants in the field; that is, the progeny of from 2 to 6 greenhouse plants.

EXPERIMENTAL RESULTS

SOIL-TEMPERATURE SERIES

Throughout the course of the experiment potatoes were grown with mean soil temperatures ranging from 55° to 85° F. In producing the tubers of the soil-temperature lots which were tested in both 1922 and 1923, the soil moisture was maintained at two extremes,

high and low, or between 90 and 100 per cent capacity in the one series and between 40 and 50 per cent in the other. (Table 1.) The tubers represented in the 1924 and 1926 trials were grown in soils with a high soil-moisture content.

The soil temperature exerted a very decided effect upon the appearance of the tubers produced. The low-temperature (55° to 65° F.) tubers were dark red to almost purple in color, whereas the high-temperature (75° to 85° F.) tubers were light pink. The low-temperature tubers were more nearly round, having ratios of width to length varying from 1.00 to 1.25, whereas the similar ratios of the high-temperature tubers generally were less than 1.00, indicating relatively a considerable degree of elongation. Furthermore, the low temperature tubers were generally quite firm and frequently the apical cluster of eyes was on the upper surface, whereas the high-temperature tubers were sometimes soft or spongy and the apical cluster of eyes was always on the end of the tuber.

TABLE 1.—*Yields obtained in comparative field tests at Scottsbluff and Alliance, Nebr., from seed potatoes grown in the greenhouse in soils of different temperature and different moisture content*

[Mean yields expressed in pounds per plant] [a]

Mean temperature of soil at which seed tubers were produced (° F.)	Mean yields at Scottsbluff [b] in—					Mean yields at Alliance [c] in 1926: High moisture
	1922		1923		1924: High moisture	
	Low moisture	High moisture	Low moisture	High moisture		
55° F			1.94±0.093	1.65±0.069	0.87±0.19	
65° F	1.48	1.68	1.90± .069	1.60± .052		0.49±0.009
70° F	1.77	1.82			2.20± .016	
71.5° F					2.04± .021	
75° F	1.73	1.90	1.61± .024	1.67± .094		.48± .084
85° F			1.49± .053	1.60± .067		

[a] The formula used for calculating the probable error of the mean was $\frac{0.6745\sigma}{\sqrt{n}}$. The 1922 data were obtained in a manner that did not permit the calculation of the probable error.
[b] Under irrigation.
[c] On dry land.

The lowest temperatures caused the plants to mature about four weeks later than the higher temperatures. Consequently, due to the difference in length of time permitted for rest periods, a variable factor was unavoidably introduced.

In the comparative trial plots in western Nebraska the plants from the high-temperature tubers sometimes emerged a few days later and seemed to produce fewer stems per plant than the tubers from the other treatments.

Although the soil temperature very materially affected the shape and color of the tubers it did not affect the productivity of the seed potatoes. (Table 1.) If the probable errors are taken into consideration it is apparent that only a few of the yields showed any statistically significant difference. These differences were inconsistent, having been in favor of the low-temperature sets in 1923 and the high-temperature sets in 1922.

SOIL-MOISTURE SERIES

With high soil moisture the life of the plants was prolonged and the tubers produced were somewhat more elongated than with low soil moisture. Data presented in Tables 1 and 2 reveal no statistically significant differences between the yields secured from "high" and "low" moisture tubers.

TABLE 2.—*Yields obtained in comparative field tests at Scottsbluff and Alliance, Nebr., from seed potatoes grown in the greenhouse in soils of different moisture content*

[Mean yields expressed in pounds per plant]

Moisture content of soil in which seed tubers were produced	Mean yields at Scottsbluff in—		Mean yields at Alliance in 1926
	1921	1922	
Per cent of capacity:			
30	1.56		0.369±0.017
45	1.78		
50		1.39±0.119	
60	1.64	1.27± .099	
70		1.32± .109	.472± .010
75	1.68		
80		1.45± .088	
90	1.73	1.39± .103	.463± .012
100	.96	1.31± .105	

In 1927 the most thorough comparative tests were made at Alliance, Nebr. These are reported in Tables 3 and 4. Two lots of seed were used. Lot A contained plants that varied greatly with regard to time of maturity, whereas the plants in lot B were very uniform and appeared to be earlier and somewhat less vigorous. The high-moisture lots were given an amount of moisture known to be excessive, the medium-moisture lots were supplied with what appeared to be an optimum amount, while the low-moisture lots were given just enough to keep the plants alive. On June 7, 10 seed pieces were planted from each greenhouse plant. As nearly as possible 10 seed pieces were cut out of 250 gm. of tubers. Due to the small size of some of the tubers it was necessary to reduce the weight of seed planted in the extreme-moisture sets of tubers. (See Table 4.) Triumph potatoes from the original stock of lot A were used as checks, 10 seed pieces per 450 gm. Checks were planted in the rows on each side of the trial stocks.

TABLE 3.—*Emergence of plants from seed potatoes grown in the greenhouse in soils of different moisture content, Alliance, Nebr., 1927*

Moisture content of soil in which seed tubers were produced	Per cent of plants emerged on date indicated and number of days required					
	June 27; 20 days	June 30; 23 days	July 4; 27 days	July 8; 31 days	July 11; 34 days	July 14; 37 days
High	14.5	30.0	79.0	84.5	90.5	90.5
Medium	25.5	55.5	92.0	96.0	97.0	97.0
Low	46.5	76.5	98.5	99.5	99.5	99.5
Checks	80.0	91.5	98.0	98.8	99.2	99.2

TABLE 4.—*Comparative yields from seed potatoes grown in the greenhouse in soils of different moisture content, Alliance, Nebr., 1927*

[Each mean represents 8 plots of 10 hills each]

SEED LOT A[a]

Moisture content of soil in which seed tubers were produced	Mean weight per seed piece planted (grams)	Mean total yield as percentage of mean of adjacent checks	Yield increase, in comparison with yield from low-moisture seed		Yield decrease, in comparison with yield from medium-moisture seed	
			Per cent of checks	Ratio of increase to probable error	Per cent of checks	Ratio of decrease to probable error
High	22.4	97.9±4.76	3.5±5.4	0.7:1	7.8±5.2	1.52:1
Medium	25.0	105.7±1.96	11.3±3.3	3.5:1		
Low	24.0	94.4±2.61			11.3±3.3	3.46:1

SEED LOT B[b]

High	17.0	82.9±4.67	2.7±4.9	0.5:1	3.4±5.3	0.7:1
Medium	24.8	86.3±2.60	6.1±3.1	2.0:1		
Low	23.0	80.2±1.63			6.1±3.1	2:1

[a] The mean yield of checks for lot A was 1.45±0.03 pounds per plant (241.6 bushels per acre).
[b] The mean yield of checks for lot B was 1.63±0.019 pounds per plant (271.7 bushels per acre).

The high-moisture seed plants emerged most slowly and the low-moisture series most rapidly. (Table 3.) The early maturity of the low-moisture plants in the greenhouse provided these potatoes with more time to rest before planting. Consequently they had more thoroughly emerged from their dormant period. None, however, were as completely through dormancy as the checks. The difference in time of emergence was soon overcome by the plants, and several weeks after planting it was impossible to detect any difference in the vigor or size of vine growth.

The yield of each 10-hill plot was compared with the mean of the two adjacent checks. The mean of these percentage relations is presented in Table 4. On this basis, the yields of the medium-moisture lots were highest. However, the difference in the mean weight of seed pieces was sufficient to account for some of this difference.

The checks were most productive because of the larger seed pieces planted. The higher yield of the checks among lot B was due to the weaker vine growth of that lot, and the consequent competitive advantage given the check plants.

SOIL-TEXTURE SERIES

The texture of the soil had a very pronounced effect upon the appearance of the tubers produced. On the heavy soils the plants matured later and the tubers were longer and darker colored than on the light sandy soils.

TABLE 5.—*Field performance of seed potatoes grown in the greenhouse in soils of different texture, Scottsbluff, Nebr., 1924 and 1925*

COMPARATIVE TRIALS IN 1924

Type of soil in which seed tubers were produced	Number of plants in trial	Mean yield per plant (pounds)	Mean relative tuber type *
80 per cent sand, 20 per cent silt	48	1.92±0.124	6.4
40 per cent sand, 60 per cent silt	48	1.36± .098	5.5
100 per cent silt	50	.76± .148	4.3

COMPARATIVE TRIALS IN 1925

100 per cent garden soil	26	2.18±0.88	
75 per cent garden soil, 25 per cent compost	30	2.66± .218	
50 per cent garden soil, 50 per cent compost	36	2.12± .184	

* Mean relative type based on type valuation from 1 to 10 for each individual tuber.

No effect upon the seed value could be determined on the basis of comparative trials. (Table 5.) In the case of the 1924 series the lot without sand remained green for a long time and became infected with spindle tuber from some plants grown in the same greenhouse (aphids not having been adequately controlled at the end of the season). Consequently the mean type of the tubers of that line was quite poor and the yield was low. By prolonging the life of the plants fertile or moisture-retaining soils may increase the opportunity for infection by disease.

DISCUSSION

The prolonging of plant life due to low temperature, high moisture, or rich soils may indirectly exert a considerable influence upon potato seed stocks (1) by extending the time the plants are exposed to virus-disease infection and (2) by allowing the tubers a shorter rest period than may be advisable. This latter condition may be of practical significance in the case of potatoes grown in the North for planting in the Southern States during December and January, before all of the tubers have passed through their dormant period under customary storage conditions.

SUMMARY

Normal healthy potato seed stocks produced in soil at different temperatures and of varying moisture content and texture were planted in the field to determine whether (or to what extent) such environmental factors exerted an influence upon their productivity.

It was found that while the soil temperature did materially affect the shape and color of the seed tubers it had no significant influence upon their productivity.

The yields from seed stocks produced upon soil containing high, medium, and low amounts of moisture were not sufficiently different to be of statistical importance.

While soil texture had a very pronounced effect upon the appearance of the tubers produced, no definite and consistent effects upon the yield produced from those tubers could be deduced from the results of the comparative field trials.

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